Evaluation of two repellent semiochemicals for disruption of attack by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae)

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ABSTRACT

When released from attractant-baited multiple-funnel traps, 3-methyl-2-cyclohexen-1-one (MCH) reduced catches of male and female mountain pine beetles, *Dendroctonus ponderosae* Hopkins, by 67.4% and 71.8%, respectively. 2-Phenyl ethanol reduced the respective catches by 96.6% and 95.1%, but only verbenone and all three compounds together reduced catches to levels no different from those in unbaited control traps. In another experiment, all three binary combinations of the above compounds, plus the ternary combination, reduced catches of both sexes by >96%. In comparable tree protection experiments near Princeton BC, MCH and 2-phenyl ethanol alone and together significantly reduced the percentages of pheromone-baited lodgepole pines that were attacked by 16.0%, 33.3% and 40.0%, respectively, but verbenone alone totally protected baited trees, and many trees within 5 m of them, from attack. In identical experiments near Prince George BC, where mountain pine beetle populations were much higher, adding MCH, 2-phenyl ethanol or both together to verbenone did not cause attack to be reduced significantly beyond that achieved by verbenone alone. Our results confirm that 2-phenyl ethanol is an antiaggregation pheromone for the mountain pine beetle, and that MCH is an interspecific synomone. However, because neither was as effective as verbenone in protecting pheromone-baited trees from attack, and adding either or both to verbenone did not improve protection, neither compound warrants further consideration as a potential tool for operational disruption of attack.

Key Words: *Dendroctonus ponderosae*, semiochemicals, pheromones, verbenone, 2-phenyl ethanol, 3-methyl-2-cyclohexen-1-one, attack disruption

INTRODUCTION

Although the antiaggregation pheromone verbenone has long been known to disrupt attack by the mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins (Amman *et al.* 1989; Lindgren *et al.* 1989a), its efficacy has been inconsistent between years, target species of trees, and geographic areas (Bentz *et al.* 1989; Lister *et al.* 1990; Gibson *et al.* 1991; Shea *et al.* 1992). Part of the reason for variable efficacy may be that verbenone is transformed to the inactive compound chrysanthenone when exposed to ultraviolet radiation (Kostyk *et al.* 1993). Adding repellent nonhost volatiles from angiosperm tree bark to verbenone has been shown to increase the efficacy of protecting lodgepole pines, *Pinus contorta* var. *latifolia* Engelmann, from attack (Huber and Borden 2001), and combining a seven-component nonhost

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volatile blend with an increased release rate of verbenone has raised the efficacy even higher (Borden *et al.* 2003). However, at an effective 10 x 10 m spacing, the latter treatment would cost $1,250 per ha, excluding labor, limiting its potential use.

One means of reducing the cost would be to replace the repellent nonhost volatile blend with cheaper materials. Two such semiochemicals are the antiaggregation pheromone 2-phenyl ethanol (Pureswaran *et al.* 2000) and 3-methyl-2-cyclohexen-1-one (MCH). MCH is an antiaggregation pheromone of Douglas-fir and spruce beetles, *Dendroctonus pseudotsugae* Hopkins and *D. rufipennis* (Kirby), respectively (Rudinsky *et al.* 1972; Lindgren *et al.* 1989b) that was recently shown to be a repellent synomone for the mountain pine beetle (Pureswaran and Borden 2004). Our objectives were to confirm the bioactivity of 2-phenyl ethanol and MCH, and to determine in trapping and tree protection experiments whether they are potential adjuvants that could increase the efficacy of verbenone.

MATERIALS AND METHODS

Two randomized complete block, 12-replicate, field trapping experiments (Exp. 1 and 2) were set up on 31 July and 13 August 2002, respectively, near the East Gate of Manning Park BC (49° 19' N, 120° 35' W). Tree protection experiments (Exp. 3A and 4A) with treatments identical to those in the trapping experiments were set up on 5-7 July 2002 in the valley of Whipsaw Creek near Princeton BC (49° 9' N, 120° 41' W), and two additional identical experiments (Exp. 3B and 4B) were set up on 24-27 July 2002 on the 1400 Road south of Prince George BC (53° 21' N, 123° 10' W).

For trapping experiments, 12-unit multiple-funnel traps were deployed at least 15 m apart along logging roads that passed near infested stands. For the tree protection experiments, lodgepole pines with a minimum diameter at breast height (dbh = 1.3 m) of 20 cm were selected at least 25 m apart in rows at least 50 m apart through cut blocks designated for harvest in the fall of 2002.

Treatments (Tables 1, 2) in Exp. 1, 3A and 3B were an unbaited trap or tree (negative control), and an attractive bait alone (positive control) or with MCH, 2-phenyl ethanol, verbenone, or all three together. In Exp. 2, 4A and 4B, the control treatments were the same, but the three disruptants were deployed in all three possible binary blends and the ternary blend. All semiochemicals and release devices were purchased from Phero Tech Inc., Delta BC. The attractive trap bait consisted of the host kairomone myrcene released from a 20 mL polyethylene bottle at 95 mg/24 h, determined at 23 °C, and the aggregation pheromones 82% (-)-*trans*-verbenol and (±)-*exo*-brevicomin respectively released from bubble caps and polyurethane flexlures at 1.2 and 0.3 mg/24 h, determined at 20 °C. The attractive tree bait was identical to the trap bait, but with myrcene deleted (Borden *et al.* 1993). MCH, 2-phenyl ethanol and 80% (-)-verbenone were released from bubble caps at 4.0, 4.2 and 1.8 mg/24 h, determined at 20, 25, and 20 °C, respectively. Devices were hung in the central funnel of traps and affixed to the north face of trees at maximum reach from the ground. The dbh of all baited trees was measured, and varied among experiments (mean ± SE) as follows: 30.9 ± 1.3 cm to 35.1 ± 1.2 cm in Exp. 3A and 4A near Princeton, and 23.0 ± 0.5 cm to 25.3 ± 0.9 cm in Exp. 3B and 4B near Prince George.

Captured beetles in Exp. 1 and 2 were collected on 13 and 26 August, respectively. Beetles were held at ca. -5 °C in plastic bags until sexed and counted.

Tree protection experiments were evaluated on 25-27 September (Exp. 3B and 4B) and 3-4 October (Exp. 3A and 4A). The attack density was counted at eye level in two 20 x 40 cm panels on the east and west faces of baited trees and all trees

Table 1.
Effect of MCH, 2 phenyl ethanol and verbenone alone or in binary or ternary combinations, on catches of mountain pine beetles in attractant-baited multiple-funnel traps. Eastgate Road near Manning Park, B.C., 31 July – 13 August 2002 for Experiment 1 and 13 – 26 August for Experiment 2.

Exp. No.	Treatment[1]	No. reps	Mean number of beetles captured (±SE)[2]	
			Males	Females
1	MPB bait	12	137.8 ± 47.4 a	86.5 ± 38.8 a
	Bait + MCH	12	44.9 ± 8.9 b	24.4 ± 8.2 b
	Bait + 2 PE	12	4.7 ± 1.1 c	4.3 ± 1.2 c
	Bait + V	12	3.5 ± 1.2 cd	2.3 ± 1.5 cd
	Bait + V + MCH + 2PE	12	1.8 ± 0.5 cd	1.1 ± 0.8 d
	Unbaited	12	0.8 ± 0.4 d	0.8 ± 0.3 d
2	MPB bait	12	76.1 ± 29.8 a	41.3 ± 16.0 a
	Bait + MCH + 2PE	10	1.7 ± 0.7 b	1.6 ± 0.7 b
	Bait + V + MCH	12	2.2 ± 1.5 b	1.1 ± 0.6 b
	Bait + V + 2PE	12	0.5 ± 0.2 b	0.2 ± 0.2 b
	Bait + V + MCH + 2PE	12	0.4 ± 0.2 b	0.6 + 0.2 b
	Unbaited	12	0.1 ± 0.1 b	0.3 ± 0.1 b

[1] Treatments as follows: MPB bait = mountain pine beetle bait including *trans*-verbenol, *exo*-brevicomin and myrcene; MCH= 3-methyl-2-cyclohexen-1-one; 2PE= 2-phenyl ethanol; V=verbonene.
[2] Means followed by the same letter are not significantly different, REGW test, P<0.05 ANOVA results: Exp. 1., males, F=53.87, df=5,66, P<0.0001; Exp. 1, females, F=34.89, df=5,66, P<0.0001; Exp. 2, males, F=14.45, df=16,52, P<0.0001; Exp. 2, females, F=9.84, df=16,52, P<0.0001.

with at least five attacks in the total 0.16 m^2 area (31.25/m^2) were classed as mass-attacked. All surrounding lodgepole pines at least 17.5 cm dbh within 5 m of baited trees were evaluated as unattacked, attacked, or mass-attacked, the latter being determined qualitatively by visual estimation of attack density and copious amounts of frass in bark crevices and around the root collar.

Data for numbers of beetles captured and attack density on baited trees were log-transformed and analyzed by ANOVA and the REGW test (Day and Quinn 1989). Data on proportions of baited and surrounding trees that were mass-attacked were analyzed by chi-square tests for comparison between multiple proportions (Jones 1984). In all cases $\alpha = 0.05$.

RESULTS

In the first trapping experiment (Exp. 1), MCH, 2-phenyl ethanol and verbenone reduced the catches of male and female mountain pine beetles in attractant-baited traps by 67.4%, 71.8% and 96.6%, and 95.1%, and 97.5% and 97.3%, respectively, relative to catches in baited control traps (Table 1). 2-Phenyl ethanol reduced catches to levels no different from those achieved by verbenone (both sexes) or the ternary blend (males only), but only the latter two treatments resulted in catches not significantly different from those in unbaited control traps. In Exp. 2, all binary

Table 2.
Effect of treatment with MCH, 2-phenyl ethanol and verbenone alone, or in binary or ternary combinations, on ranked percentages of pheromone-baited lodgepole pines that were mass-attacked, and on the pooled percentages of all surrounding trees ≥ 17.5 cm dbh within 5 m of the baited tree that were mass-attacked.

Exp. no. (no. reps)	Location	Treatment[1]	Percent baited trees mass-attacked[2]	Surrounding trees	
				N	Percent mass-attacked[2]
3A (25)	Princeton	MPB bait	100.00 a	103	15.5 ab
		Bait + MCH	84.0 b	119	23.5 a
		Bait + 2PE	66.7 b	104	4.8 b
		Bait +V + MCH + 2PE	0.0 c	127	0.0 c
		Unbaited	0.0 c	100	0.0 c
		Bait + V	0.0 c	117	0.0 c
3B(20)	Prince George	MPB bait	95.0 a	69	47.8 ab
		Bait + MCH	80.0 ab	83	31.3 ab
		Bait + 2PE	75.0 abc	62	24.2 b
		Unbaited	40.0 bc	65	49.2 a
		Bait +V + MCH + 2PE	40.0 bc	75	18.7 b
		Bait + V	35.0 c	70	27.1 ab
4A (20)	Princeton	MPB bait	100.0 a	96	26.0 a
		Bait + MCH + 2PE	60.0 b	93	15.1 a
		Bait + V + MCH	5.3 c	87	1.1 b
		Bait + V + 2PE	0.0 c	89	0.0 b
		Unbaited	0.0 c	103	0.0 b
		Bait + V + MCH + 2PE	0.0 c	105	0.0 b
4B (17)	Prince George	MPB bait	94.1 a	52	44.2 a
		Bait + V + MCH + 2PE	47.1 b	47	25.5 ab
		Bait + V + 2PE	35.3 b	61	23.0 ab
		Unbaited	29.4 b	67	17.9 b
		Bait + MCH + 2PE	23.5 b	52	36.5 ab
		Bait + V + MCH	17.6 b	51	25.5 ab

[1] Treatments as in Table 1, Footnote 1, except that myrcene is not present in MPB bait.
[2] Percents within a column and experiment followed the same letter are not significantly different, chi-square test for multiple proportions, $P<0.05$.

combinations and the ternary combination of disruptants reduced catches by more than 96%, and in all cases catches in traps with disruptive treatments were no greater than in unbaited control traps.

In the first tree protection experiment near Princeton (Exp. 3A), all pheromone-baited control trees were mass-attacked (Table 2). MCH and 2-phenyl ethanol alone reduced the proportion of baited trees that were mass-attacked by 16.0% and 33.3%, respectively, but verbenone and the ternary blend completely protected pheromone-baited trees and all trees within 5 m of them from attack. In Exp. 3B near Prince George, only verbenone and the ternary blend significantly reduced the percentage of baited trees that were mass-attacked, and the lowest percentages of surrounding trees that were mass-attacked occurred in the 2-phenyl ethanol and ternary blend treatments.

In the second tree protection experiment near Princeton (Exp. 4A), the binary combination of MCH and 2-phenyl ethanol reduced the percentage of baited trees that were mass-attacked by 40%, but did not cause a reduction in the proportion of surrounding trees that were mass-attacked (Table 2). All treatments containing verbenone reduced attack to zero or to a level not significantly different from zero. In Exp. 4B near Prince George, all treatments significantly and equally reduced the percentage of baited trees that were mass-attacked, but no treatment had a significant effect on attack on surrounding trees.

In all cases except Exp. 3B (Bait + V + MCH + 2 PE), disruptant treatments including verbenone caused a reduction in attack density on mass-attacked trees relative to the MPB bait alone, but in the absence of verbenone, only MCH + 2-phenyl ethanol in Exp. 4B caused a similar reduction (Table 3).

Table 3.
Effect of treatment with MCH, 2-phenyl ethanol and verbenone alone, or in binary or ternary combinations, on ranked densities of attack by the mountain pine beetle on pheromone-baited lodgepole pines.

Exp. no. (no. reps)	Location	Treatment[1]	No. attacked trees	Mean attack density/ $m^2 \pm$ SE on attacked trees[2]
3A (25)	Princeton	MPB bait	25	125.5 ± 10.1 a
		Bait + 2PE	16	112.5 ± 13.8 a
		Bait + MCH	23	108.1 ± 7.6 a
		Bait + V + MCH + 2PE	3	6.3 ± 0.0 b
		Bait + V	0	no attack
		Unbaited	0	no attack
3B (20)	Prince George	Bait + MCH	15	105.4 ± 10.5 a
		MPB bait	19	91.4 ± 8.5 ab
		Bait + 2PE	14	77.3 ± 10.4 ab
		Unbaited	10	62.5 ± 13.8 bc
		Bait + V + MCH + 2PE	8	60.9 ± 7.6 bc
		Bait + V	13	52.9 ± 10.2 c
4A (20)	Princeton	MPB bait	20	129.1 ± 8.4 a
		Bait + MCH + 2PE	14	98.7 ± 14.8 a
		Bait + V + MCH	6	25.0 ± 11.9 b
		Bait + V+ 2PE	5	8.8 ± 4.3 b
		Bait + V + MCH + 2PE	6	8.3 ± 3.5 b
		Unbaited	1	no attack
4B (17)	Prince George	MPB bait	15	116.3 ± 9.1 a
		Bait + V + MCH + 2PE	11	64.2 ± 40.1 b
		Bait + V + 2PE	9	58.3 ± 11.3 b
		Unbaited	9	43.8 ± 10.8 b
		Bait + MCH + 2PE	12	40.6 ± 13.9 b
		Bait + V + MCH	7	40.2 ± 14.8 b

[1] Treatments as in Table 1, Footnote 1, except that myrcene was not present in MPB baits.
[2] Percents within a column and experiment followed the same letter are not significantly different, chi-square test for multiple proportions, $P<0.05$.

DISCUSSION

Our results confirm the bioactivity of 2-phenyl ethanol as an antiaggregation pheromone of the mountain pine beetle (Pureswaran *et al.* 2000), and MCH as a repellant synomone (Pureswaran and Borden 2004).

Unlike MCH, 2-phenyl ethanol appeared to have some potential as a pest management tool. MCH caused only marginal reductions in trap catches, and afforded little protection of trees. In contrast, 2-phenyl ethanol caused large reductions in trap catches and greater protection of pheromone-baited and surrounding trees. In Exp. 1, 2, 3A and 3B in the southern part of the province, verbenone was so effective alone that there was no opportunity to observe any potential interaction between MCH, 2-phenyl ethanol and verbenone. However, near Prince George, where beetle pressure was much higher than near Princeton, adding MCH, 2-phenyl ethanol or both together to verbenone did not cause any greater protection of pheromone-baited or surrounding trees than was achieved by verbenone alone. In a similar experiment nonhost volatiles added to verbenone resulted in substantially greater protection of baited and surrounding trees than was found with verbenone alone (Huber and Borden 2001). However, in a small plot experiment the same nonhost volatile blend caused a greater reduction of attack than verbenone alone when added to high-dose verbenone pouches, but not when added to the same low-dose bubble caps used in our experiments (Borden *et al.* 2003).

Our results show that when tested alone, 2-phenyl ethanol was more effective than MCH in reducing catches in attractant-baited traps (Table 1), as well as the percentage of baited and surrounding trees that were mass-attacked (Table 2, Exp. 3A). However, neither MCH nor 2-phenyl ethanol alone reduced the attack density of trees that were mass-attacked (Table 3), and there was no apparent additive or synergistic effect of combining the two compounds or of adding them alone or together to verbenone to protect trees from attack. Therefore, we conclude that neither compound has compelling potential for use as an adjuvant to verbenone in operational attack disruption programs.

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